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REVIEW ARTICLE

Proteomic impacts of electromagnetic fields on the male reproductive system

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Abstract The use of mobile phones and other wireless transmitting devices is increasing dramatically in developing and developed countries, as is the rate of infertility. A number of respected infertility clinics in Australia, India, USA, and Iran are reporting that those who regularly use mobile phones tend to have reduced sperm quantity and quality. Some experimental studies have found that human sperm exposed to electromagnetic fields (EMF), either simulated or from mobile phones, developed biomarkers of impaired structure and function, as well as reduced quantity. These encompass pathological, endocrine, and proteomic changes. Proteins perform a vast array of functions within living organisms, and the proteome is the entire array of proteins—the ultimate biomolecules in the pathways of DNA transcription to translation. Proteomics is the art and science of studying all proteins in cells, using different techniques. This paper reviews proteomic experimental and clinical evidence that EMF acts as a male-mediated teratogen and contributor to infertility.

Keywords Proteome · Mobile phone · Electromagnetic · Male-mediated teratogen · Blood-testis-barrier · Heat-shock proteins

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Introduction

The capacity of various exogenous factors to disrupt normal growth and development can be demonstrated with in vitro and in vivo experimental techniques, and with clinical and epidemiological studies. The male-mediated teratogens have been used to describe different environmental factors such as cocaine, alcohol, certain pesticides, and heavy metals. They can affect the valency of men to become fathers and also the health of their offspring (Davis et al. 1992). Not surprisingly, these same agents also act as direct teratogenic factors that work via classical routes of raised placental uptake. Where dominant lethality does not occur, survival of damaged offspring can result in reduced numbers of animals, and in humans can lead to children with various structural or functional impairments.

Electromagnetic fields (EMF) induce a range of damaging impacts on the capacity of males to produce healthy offspring, affecting pre- and peri-fertilization, as well as producing teratogenic results including miscarriage. At conception, males bring almost an equal number of chromosomes to their progeny and their genomes may touch various situations of reproduction. Many peoples believe that the key male role principally ends at fertilization, but there is growing empirical and human documents that describe how pre- and peri-fertilization exposure also plays a role post-fertilization. Currently, there is a lack of human epidemiologic findings regarding the importance of male-mediated teratogenesis, reflect that collected information usually generates detailed descriptions of maternal exposures and do not collect evidences about pre- or peri-fertilization paternal exposures. The absence of extensive human evidence on the male reproductive impacts caused by

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EMF exposure should be interpreted as a deficiency in research which needs to be corrected by more researches.

The EMF-related effects on the male genital systems can be categorized as either structural, including pathophysiological and expressional, or functional, including histological, proteomic, and endocrine. Vulnerability to toxic insult can be thought to vary with the rate of cell division. This means that rapidly dividing cells, such as those of spermatocytes and embryos, will be especially susceptible. The faster growth and younger cells, the greater their propensity to develop, incorporate, and sustain errors and the lesser the cells' ability to repair damage. Here, we review historical and recent studies about adverse effects of EMF exposure on the proteonomics of the male reproductive system.

EMF: nature and producing apparatus

Electromagnetic radiation, as a fundamental force of nature, is comprised of two intersecting components: an electric field produced by static charged objects and a magnetic field which is created by moving charges (Cucurachi et al. 2013). These waves have different names based on their wavelength, including very low and low frequency, radiofrequency or microwave, infrared, visible light, ultraviolet, X-ray, and gamma ray. These waves can be envisioned in two distinct ways: a continuous wave, with energy being transferred continuously between any two locations or a discrete model in which energy is being carried in the form of quanta (photons) with a fixed frequency. Our atmosphere is filled with unnatural EMF from various man-made equipment such as power lines and electrical equipment (e.g., air conditioners), transmission antennae, mobile phones, computers, and Wi-Fi. Wireless devices such as mobile phones are most important because men routinely use them close to their head for talking, and near their genitals when using head-sets and other wireless devices, or when carrying the devices in their pockets when not in use (Fig. 1) (Sepehrimanesh et al. 2014b). Effects may be either functional, resulting in changes of hormones or other important biochemicals of the living system or structural, resulting in physical changes in the cell's and organelles membranes.

Therefore, the genital system may be most at risk. We review both historical and recent effects reported in historical and recent literature. First, however, we describe the proteomic vocabulary and principle genital and related systems. We concurrently discuss mobile phone-related adverse effects in light of proteomic changes.

Proteomics

As the large-scale study of proteins, proteomics evaluates the relationship between protein structure and function. The

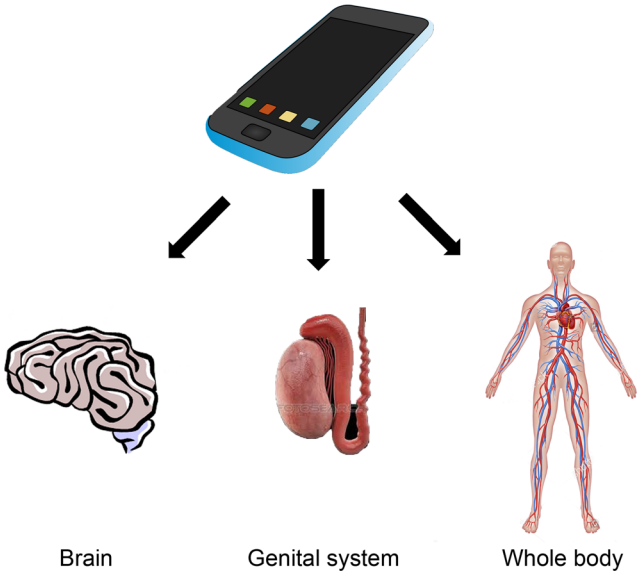

Fig. 1 Exposure from a mobile phone can affect the entire body, especially the genital system and brain

proteome varies over time as well as according to requirements (e.g., reproduction) or stresses (e.g., temperature or nutrition) the organism undergoes. Proteins are the ultimate functional molecules in the cells, and evaluation of their identities, concentrations, changes, and modification in response to environmental factors can be valuable for all of biological science (Sepehrimanesh 2016). All the whole proteins in the cell, tissue, or microorganism, which is called the proteome, may be evaluated by various techniques including one- or two-dimensional electrophoresis (1-DE or 2-DE), western blotting, high-performance liquid chromatography (HPLC), fast protein liquid chromatography (FPLC), mass spectroscopy (MS), and so forth (Malmstrom et al. 2016). The most important techniques in this approach include the following (Boja and Rodriguez 2012; Gregorich and Ge 2014; Miller et al. 2014; Tsiatsiani and Heck 2015; Yang et al. 2015):

- 1-DE that separates the proteins of each sample only based on one propertie.
- 2-DE combines isoelectric focusing and SDS electrophoresis sequentially in a process called 2-DE which permits the resolution of complex mixtures of proteins.
- Difference gel electrophoresis (DIGE) is a form of gel electrophoresis in which a protein sample is mixed with fluorescent dyes such as Cy3, Cy5, and Cy2 prior to 2-DE.
- High-performance liquid chromatography/mass spectroscopy (LC/MS) involves chromatographic separation of mixture components and subsequent identification using MS. Matrix-assisted laser desorption ionization-time- of-flight mass spectroscopy (MALDI-TOF/MS): Historically, MS was utilized as a highly complex method

adapted for analysis of samples in chemistry and hematology laboratories.
- Stable isotope labeling by amino acids in cell culture (SILAC) is a simple method for in vivo incorporation of labeled amino acids into proteins for MS-based quantitative proteomics. By using heavy isotope labeled amino acids, heavy homologues are introduced into the approximately complete proteome during synthesis and cell replication.

Proteomic changes of the male reproductive system in response to mobile phone exposure

The male reproductive system includes four main parts: genital cells and their producing tissues, accessory glands, ducts, and the external genital system. Among the structural and functional components that may be affected by EMF produced by mobile phones are blood testes barrier, testes and semen, and accessory glands.

Blood testes barrier

The blood testes barrier (BTB) is a physical barrier between the blood vessels and the seminiferous tubules of the animal or human testes. The Sertoli and germ cells cover the walls of seminiferous tubules, and the BTB is created by certain junctions such as tight, adherence and gap junctions between the Sertoli cells (Barrett et al. 2012). Changes to the BTB proteins in response to EMF were the objective of some limited recent studies. A significant increase in the expression of tumor growth factor (TGF)-β3, a key molecule involved in BTB permeability, was reported by Luo and colleagues in response to electromagnetic pulse exposure (Luo et al. 2013). Opposite findings were reported about TGF-β3 (Wang et al. 2010) and other tight junction proteins, ZO-1 and Occludin, in response to electromagnetic pulse exposure which may change the structure and function of barrier (Qiu et al. 2011). These two studies were preliminary and could not answer that why tight junction proteins show different changes in different organs. Also, in the Wang et al.'s study, the expression level of occludin did not change after EMF exposure. It seems that EMF can alter the expression of proteins in the tight junction, especially ZO-1, and affect the testes function or induce infertility.

Testes and semen

The testes are the male gonad in animals and humans, and they produce semen which is comprised of sperm and seminal plasma. Numerous controlled studies have reported the adverse effects of EMF on testicular tissue and semen; however, protein and proteomic related studies are less frequent. The effects of in vivo and in vitro exposure to EMF on testicular tissue and semen were reviewed completely by Makker et al., in 2009. They also reported changes in the transcriptomics and proteomics of different cells except genital cells in human and animal tissues (Makker et al. 2009). Conflicting findings have been obtained, although the circumstances of exposure are not always consistent. For instance, Sepehrimanesh et al. evaluated the rat testicular weight and protein content by Bradford method after different exposure times to 900 MHz EMF. They found no statistically significant differences between sham and exposed groups, neither in the testicular protein concentration nor the weight of testes (Sepehrimanesh et al. 2014a). Parivar et al. reported that 100 Hz EMF exposure for 7 h twice a day for two consecutive days did not induce significant changes in mice weight and testes weight. But the number of spermatogonia and secondary spermatocyte showed a significant increase in response to EMF exposure. Other than genital effects, they found that the percentage of alpha-globulins in serum significantly decreased in exposed mice (Parivar et al. 2011). No significant changes in lipid peroxidation sperm count and level of p53 in testicular tissue of rats in response to extremely low-frequency EMF (ELF-EMF) as 2 h/day for 2 months (7 days a week) were reported (Akdag et al. 2006). Ozguner and collaborators in their study about the effect of electromagnetic fields on undescended testis after orchiopexy concluded that EMF stimulation proliferated Leydig cells, increased testosterone level and testicular weight, but decreased the population of germ cells (Ozguner et al. 2002).

Down-expression of lipocalin 2, a protein which mediates delivery of ferric ions to mouse spermatozoa and enhances sperm motility (Mohammadi Roushandeh et al. 2009), and metallothionein (MT) 1 and 2, inducible forms of MTs which their transcription is activated by a variety of stress stimuli, by real-time PCR and western blotting in mouse testis after exposure to EMF was reported. Recently, it has been confirmed that the expression of proteins related to the misfolding of proteins and/or stress such as heat shock proteins, superoxide dismutase, and peroxiredoxin-1 were changed in response to continuous EMF exposure (Sepehrimanesh et al. 2014a). Akdaq and collaborators reported that long-term exposure to 100 and 500 μT ELF-EMF did not significantly change sperm count and morphology in testicular tissue of rats. However, long-term exposure to 500 μT ELF-MF changed the active-caspase-3 activity as an apoptotic indicator (Akdag et al. 2013). In a recent experimental study by Gong and collaborators, low dose gamma ray radiation decreased superoxide dismutase activity, the expressions of DNA methlytransferases-1, and histone deacetylases 1 in murine testes (Gong et al. 2014). Also, an increase in activity of xanthine oxidase and acid-DNase in rat testicular tissue was reported after exposure to microwave radiation for 4 h per day (Sokolovic et al. 2015). Kesari et al. reported tumor promotion in testicular cells by

identifying changes in the level of some important proteins such as protein kinase C and histone kinase (Kesari and Behari 2010; Kesari et al. 2010, 2011, 2013). Also, Desai et al. expressed that changes of protein kinase C, ornithine decarboxylase, and stress kinases in response to radiofrequency EMF may have had a tumor-promoting effects (Desai et al. 2009).

Apoptosis

One of the most important changes related to the EMF exposure is apoptosis, a programmed cell death. Active (cleaved) caspase-3 expression as an index of apoptosis was measured semi-quantitatively by immunohistochemistry in response to cell phone EMF exposure. The results showed that 2 h/day (7 days/week) over a period of 10-month exposure did not affect apoptosis (Dasdag et al. 2008). However, Yilmaz et al. reported that 900-MHz mobile phones cannot influenced the anti-apoptotic bcl-2 protein levels in rat testes (Yilmaz et al. 2008). Also, the effects of simultaneous combined exposure to single code division multiple access (CDMA) and wideband code division multiple access (WCDMA) RF electromagnetic signals for 12 weeks on apoptosis indices in rat testes. Although immunoblotting for p53, bcl2, GADD45, cyclin G, and HSP70 in the testes of sham- and RF-exposed animals was done, no adverse effects related to CDMA and WCDMA were detected related to these indices (Lee et al. 2012). In another study, the level of TNF-α, caspase-3, and Bcl-2 was compared between control and 2450 MHz exposed group, and no inter-group significant difference was detected. When Bax apoptosis genes and caspase-8 apoptosis enzyme were compared, in inter-group difference was statistically significant (Saygin et al. 2011).

Accessory glands and extra genital system

The adverse effects of EMF on the semen-producing organs such as accessory gland were reported. Afshari et al. reported that exposure of Wistar rats to a 50-G magnetic field for 4 h per day for 3 months induced a reduction in the epithelium height and the nuclei in the prostate. The vas deferens were condensed, sterocilia disappeared and prostatic secretions were reduced (Afshari et al. 2013). In pre-pubertal male rats exposed to a 1.95-GHz WCDMA signal for 5 h/day for 5 weeks, the weights of the testis, epididymis, seminal vesicles, and prostate were unchanged (Imai et al. 2011). The weight of seminal vesicles and prostate was significantly higher in male offspring of pregnant rats field-exposed to a 15-Hz pulsed EMF for 15 min twice a day from day 15 through day 20 of gestation, compared with control animals (McGivern et al. 1990). However, there are no reports identifying a protein or proteome changes in male accessory glands and extra genital system in response to electromagnetic exposure.

Conclusion

As among the most rapidly proliferating human cells, spermatogenesis and associated activities offer an important endpoint for evaluation. More than 60 different compounds or industrial processes have been identified as increasing defects in human sperm or testicular tissue and possibly increasing the risk to offspring from male-mediated exposures. In this study, we reviewed structural and functional proteomic changes related to EMF exposure. Reported changes are categorized based on main affected tissue and also the most important adverse effects. Overall, these results demonstrate significant effects of radio frequency-modulated EMF exposure on the proteome, including both structural and functional impacts such as a decrease in the diameter and weight of the seminiferous tubules and the mean height of the germinal epithelium (Ozguner et al. 2005) and/or pathological and physiological changes in key biochemical components of the testicular tissues (Luo et al. 2013). These structural and functional changes may account for the pathological impact of EMF on the male reproductive system reported in the experimental work that we and others have conducted. While EMF is currently being used for a number of therapeutic applications (REF), the work we have reviewed here clearly indicates a range of harmful effects, especially on genital systems.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Due to the nature of review paper, no informed consent was available.

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